DOCKET NO. JBP0438US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Miri Seiberg et al.	Confirmation No. 5255
Serial No.	:	09/206,249	Art Unit: 1655
Filed	:	December 7, 1998	Examiner: Michael V. Meller
For	:	**METHODS FOR REGULATING PHAGOCYTOSIS AND ICAM-1 EXPRESSION**	

DECLARATION OF CONNIE BAOZHEN LIN, PH.D.

1. I, Connie Baozhen Lin, am a Senior Research Fellow in the Skin Research Center, Skin biology group, at Johnson & Johnson Consumer Companies, Inc. My education includes a Ph.D. degree in Biochemistry, M.S. and B.S. degrees in Pharmaceutical Sciences, and my *curriculum vitae* is attached hereto as Exhibit 1.

2. Phagocytosis is the cellular process of ingestion of particulate material. The above captioned patent application, relates to the prevention and treatment of mammalian disorders that are ameliorated by altering or inhibiting the phagocytosis process. The avoe captioned patent application, which is based on the discovery of a mechanism for the regulation of phagocytosis, provides numerous compositions, including those of non-denatured soybean extracts, which address a range of disorders that are affected by phagocytic processes.

3. The non-denatured soybean extracts of the above captioned patent application contain all the ingredients of the soybean in their natural conformations, which is different from soy foods. Non-denatured soybean extracts cannot be used as dietary ingredients. As summarized in e.g. (KeShun Liu (ed.). Soybeans, chemistry, technology and utilization, An Aspen publication, Gaithersburg, Maryland 1999, Trypsin inhibitors, P. 48-55), soy foods cannot contain high concentrations of active soybean trypsin inhibitor (STI), because this soybean protease inhibitor blocks the action of trypsin and other enzymes needed for protein digestion. For example, studies by Limtrakul et al. (Limtrakul P, Suttajit M, Semura R, Shimada K, Yamamoto S. Suppressive effect of soybean milk protein on experimentally induced skin tumor in mice. *Life Sciences* 1993, *53*, 1591-1596) attempted to identify a safe level of soy proteins for nutritional consumption, and documented major health problems upon the ingestion of non-denatured soybean proteins.

4. A non-denatured soybean composition made in accordance with the above-captioned patent application, and two dietary soymilk preparations which are commercially available in food stores were analyzed at my direction for their effect on the phagocytic process. The results of this study, which is documented in section 5 below, demonstrate that the non-denatured soybean extract of this invention, but not dietary soymilk preparations which are commercially available in food stores, could inhibit the process of phagocytosis and therefore could be useful in the above mentioned patent application.

5. A non-denatured soybean composition was made in accordance with the above-captioned patent application, using non-denatured high sucrose Soymilk Powder # 9104 (Devansoy Inc., Rock city, IL, USA). Commercial soymilk drinks were purchased from ShopRite (Montgomery, NJ, USA) on 11/11/10. The soymilk drinks were 1) 8th continent soymilk (light original, 07DEC1095FNA JP1, from Stremicks Heritage Foods, LLC, Santa Ana, CA, USA), and 2) Silk all natural soymilk (original, 0070 TR7L5, from WhiteWave Foods, Broomfield, CO, USA). The soymilk drinks were diluted to the equivalent protein concentration as in 0.1% of non-denatured soybean powder, based on their corresponding

protein contents. STI (Soybean trypsin inhibitor type I-S, T-9003, from Sigma, St Louis, MO, USA) served as a positive control.

Phagocytosis was quantified using the Vybrantä Phagocytosis Assay (V-6694 of Molecular Probes, Eugene, OR, USA) according to manufacture instruction and to the protocol described previously (E. R. Sharlow et al., Journal of Cell Science 113, 3093-3101 (2000)). Briefly, HaCaT keratinocytes were treated with the peptide SLIGRL (20 µM) to induce phagocytosis as in Sharlow et al., with and without exposure to non-denatured soybean extracts or to each of the commercial soymilk drinks for 48 hours. Following each treatment, cells were incubated for 4 hours with 100 µl of fluorescein-labeled *E. coli* K-12 bioparticles. The non-ingested *E. coli* suspension was aspirated, and 100 µl Trypan Blue were added for 1 minute to quench extracellular fluorescence, following by measurements of the ingested fluorescence (485 nm excitation/538 nm emission) using a SpectraMaxâ Gemini microtiter plate reader (Molecular Devices Corporation, Sunnyvale, CA, USA). Each treatment was performed with six replicates.

Data shown in the table 1 are percentage of phagocytic activity relative to untreated control. T-test (treatment vs. untreated control) was performed using Excel software.

Table 1 Phagocytic activity

Treatments	Sample info	Phagocytic activity (%) relative to untreated control +/- SEM	t-test (treatment vs. untreated control)
Untreated control		100.00+/-7.57	
20 μM SLIGRL	SLIGRL (Invitrogen, Carlsbad, CA)	185.28+/-36.80	0.03
20 μM SLIGRL + 0.05% STI	Soybean trypsin inhibitor type I-S (T-9003, Sigma, St Louis, MO, USA)	117.69+/-21.20	0.41
20 μM SLIGRL + 0.1% non-denatured soy	Non-denatured High Sucrose Soymilk Powder # 9104 (Devansoy Inc., Rock city, IL, USA)	128.59+/-21.28	0.20
20 μM SLIGRL + 0.1% soymilk-1	8th continent soymilk (light original, 07DEC1095FNA JP1) (Stremicks Heritage foods, LLC, Santa Ana, CA, USA)	222.72+/-23.45	0.00
20 μM SLIGRL + 0.1% soymilk-2	Silk all natural soymilk (original, 0070 TR7L5) (WhiteWave Foods, Broomfield, CO, USA)	208.15+/-32.54	0.01

The results documented in Table 1 show that, as expected, SLIGRL enhanced phagocytosis and STI, the positive control, was able to reverse most of the SLIGRL-enhanced phagocytosis. The non-denatured soybean extract was able to inhibit SLIGLR-induced phagocytosis to about the same level as the positive control. However, the two edible soymilk preparations not only failed to inhibit phagocytosis, but they further enhanced this process. These data demonstrate the usefulness of non-denatured soybean extracts in inhibiting phagocytic processes, and the failure of soymilk drinks to provide such an inhibitory effect.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so

made are punishable by fine or imprisonment, or both, under Section 1001 of title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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2/28/2011

Date